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Gut 2008 57: 628-633 originally published online January 8, 2008
doi: 10.1136/gut.2006.103200

Expression and functional pharmacology of the bradykinin B1 receptor in the normal and inflamed human gallbladder

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Revised 31 October 2007
Accepted 4 December 2007
Published Online First 4 January 2008

ABSTRACT

Background and aims: It has recently been described that bradykinin B_2 receptors are expressed in the human gallbladder and that their activation induces a powerful contraction, especially in acute cholecystitis tissues. Here the role of the B_1 receptor in the contractility of control and inflamed human gallbladder was investigated.

Methods: Strips of human gallbladder from either acute gallstone cholecystitis or elective gastro-entero-pancreatic surgery (control) were assessed in vitro and processed for reverse transcription-PCR analysis. Cumulative concentration–response curves with the selective B_1 receptor agonist, Lys-Des-Arg^9-bradykinin, cholecystokinin and carbachol were performed in control and cholecystitis specimens.

Results: Lys-Des-Arg^9-bradykinin concentration-dependently contracted strips of control gallbladders and its motor effect was higher in inflamed gallbladders. Lys-Des-Arg^9-bradykinin-induced contraction was not altered by pretreatment with the selective bradykinin B_2 receptor antagonist, HOE140 (1 μM), the NK_1 (SR140333), NK_2 (SR48968) and NK_3 (SR142801) tachykinin receptor antagonists (all 1 μM), the muscarinic acetylcholine receptor antagonist, atropine (1 μM), and the cyclo-oxygenase inhibitor, indomethacin (5 μM). In contrast, the Lys-Des-Arg^9-bradykinin-induced motor response was significantly reduced by the selective B_1 receptor antagonist, R-715. Finally, quantitative real-time PCR analysis indicated that B_1 receptor mRNA levels were significantly higher in cholecystitis smooth muscle specimens, when compared with that observed in control tissues.

Conclusions: Bradykinin B_1 receptor has an important role as a spasmogen of human gallbladder, and selective antagonists of the B_1 receptor may represent a valid therapeutic option to control pain in patients with acute cholecystitis.

Kinins and their receptors constitute an important biological system activated by diverse stimuli such as inflammation and injury. Kinins mediate their biological effects through the activation of B_1 and B_2 receptors.[1 2] Des-Arg^9-bradykinin and Lys-Des-Arg^9-bradykinin (Lys-DBK) show high affinity for the B_1 receptor and a low affinity for the B_2 receptor, whereas the opposite happens for bradykinin and Lys-bradykinin (Lys-BK).[1] B_2 receptors are constitutively present in a large array of cells including smooth muscle, endothelial and epithelial cells. In contrast, B_1 receptors are rarely expressed in healthy tissues and their expression is classically promoted by inflammatory cytokines, growth factors, bacterial toxins or following in vitro incubation of the tissue: rabbit aorta;[3] rat urinary bladder;[4] pig coronary arteries;[5] bovine mesenteric arteries;[6] canine blood vessels;[7] human colon[8]; and mouse trachea and urinary bladder.[9] Thus, in vitro exposure of tissues has been a well-defined method to unravel responses to selective B_1 receptor agonists and antagonists.

Diverse relaxant and contractile mediators regulate the motility of the human gallbladder: vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) relax human gallbladder smooth muscle, whereas cholecystokinin (CCK) induces muscle contraction, at least in part, through the activation of preganglionic parasympathetic neurons.[10] Bradykinin has been shown to exert a motor function in guinea pig gallbladder.[11 12] Recent evidence from our group[13] showed that bradykinin, via B_2 receptor activation, produces a robust contraction of human gallbladder in vitro with a potency and an efficacy similar to those of CCK and higher than those of carbachol (CCh). Thus, the two main mediators of the contractile response of the human gallbladder seem to be bradykinin and CCK through activation of the B_2[13] and CCK receptors,[14 15] respectively. Spasms produced by gallbladder smooth muscle contractions, in the attempt to expel stones, greatly contribute to the symptoms, particularly pain, that underline biliary colic. However, the therapeutic outcome of biliary colic obtained with antimuscarinic agents is often poor.[16 17] Thus, the question of whether additional mediators may contribute to the contraction of the human gallbladder is of importance to design novel therapeutic strategies.

In this study, we have investigated the expression and the functional activation of the B_1 receptor in human isolated normal and inflamed gallbladders. The selective B_1 receptor agonist Lys-DBK was used and the mechanism of Lys-DBK-induced gallbladder motor response was studied by using a selective bradykinin B_1 (R-715)[18] and B_2 (HOE140) receptor antagonist, a combination of tachykinin NK_1 (SR 140333), NK_2 (SR 48968) and NK_3 (SR 142801) receptor antagonists,[19–21] an antimuscarinic agent (atropine) and a cyclooxygenase inhibitor (indomethacin).

MATERIALS AND METHODS

Human gallbladders

Strips (approximately 10×20 mm) from the body of human gallbladders were obtained from patients (42–78 years) undergoing cholecystectomy.

Gallbladders were subdivided into two groups. The first included gallbladders taken from 20 patients (12 males and 8 females, 47–78 years) during routine laparoscopic cholecystectomy (cholecystitis group). Major reported complaints of patients included upper right quadrant abdominal pain, and abdominal discomfort referred to as "spasm sensation" along with nausea and vomiting. Patients were diagnosed as having acute cholecystitis due to gallstone disease. In this group of patients, ultrasonographic evaluation showed a thickened gallbladder wall (18 out of 20 patients), and macroscopic analysis, performed following surgery, demonstrated a swollen viscus in the majority of patients (90%). The second group (7 males and 5 females; 42–75 years; control group) consisted of 12 patients whose gallbladders were removed during elective abdominal surgery, including colonic or gastric resection, right hepatic lobectomy and pancreatic-duodenectomy due to neoplastic disease. All patients gave their informed consent prior to surgery, and the present study was approved by the Ethical Committee of the University of Ferrara.

Organ bath studies

Twenty minutes after surgical removal, gallbladder tissues dissected from the body of control and acute cholecystitis groups were carefully cleaned and strips were placed in 5 ml organ baths containing a Krebs buffer solution [(mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, $CaCl_2$ 2.5, KCl 4.7 and glucose 11] maintained at 37°C and oxygenated (96% O_2 and 4% CO_2). Gallbladder specimens were connected to an isometric force transducer (Unirecord 7050, Ugo Basile, Milano, Italy); to record the mechanical activity, an optimal resting tension of 1.5 g was applied.[13] After an initial stabilisation period (90 min) to test the tissue viability of control and cholecystitis specimens, cumulative concentration–response curves (CRCs) were performed with KCl (10–120 mM). After an additional 90 min, CRCs with CCK (0.01 nM–3 μM), Lys-DBK (1 nM–10 μM) and CCh (10 nM–10 μM) (first CRC) were performed. Since specific experiments showed that the response to Lys-DBK obtained at 6 h was not different in tissues with or without the mucosa (data not shown), to avoid further tissue manipulation mucosa-intact strips of the body of human gallbladders taken from control and cholecystitis groups were carefully cleaned and prepared as previously described.[13]

A supplementary CRC with CCK, Lys-DBK and CCh was performed 3 h after the first CRC (second CRC). To define better the mechanisms of Lys-DBK-induced motor effects in human gallbladder, the effect of the selective B_1 receptor antagonist, R-715 (1 μM), and of the B_2 receptor antagonist, HOE140 (1 μM), were tested. In another set of experiments, the effect of the combination of the tachykinin NK_1 (SR 140333), NK_2 (SR 48968) and NK_3 (SR 142801) receptor antagonists (all 1 μM), the antimuscarinic agent, atropine (1 μM), the cyclo-oxygenase inhibitor, indomethacin (5 μM), the protein synthesis inhibitor, cycloheximide (100 μM), or their respective vehicles were tested.

Finally, in order to confirm the concomitant activation of both B_1 and B_2 receptors in inflamed human gallbladders, R-715 (1 μM) and HOE140 (1 μM) were tested, alone or in combination, against the contraction induced by Lys-BK. This peptide is a B_2 receptor agonist that, through the catalytic action of endogenous carboxypeptidase, is transformed into Lys-DBK, which binds with high affinity to the human B_1 receptor. All antagonists/blockers were added 15–45 min prior to the second CRC performed with Lys-DBK or Lys-BK in cholecystitis group specimens, except for cycloheximide that was maintained throughout the entire experiment.

Quantitative real-time PCR

Gallbladder specimens were taken immediately (30–60 min) after surgery and the mucosa layer was carefully removed. Total RNA from the mucosa or smooth muscle tissues was isolated using TRizol reagent (Invitrogen Corp., Carlsbad, Califonia, USA) according to the manufacturers' protocol. RNA samples were treated for 1 h at 37°C with DNase I (Promega Corp., Madison, Wisconsin, USA) to avoid contaminant genomic DNA. After the incubation, samples were heated to 95°C and immediately chilled on ice for DNase I denaturation. We performed reverse transcription using 5 μg of total pure RNA, 200 U of Moloney murine leukaemia virus reverse transcriptase (Invitrogen Corp.), 5 mM dithiothreitol, 50 ng of random hexamer primers (Invitrogen Corp.), 1× PCR buffer, 0.5 mM dNTPs (Invitrogen Corp.) and 3 mM $MgCl_2$. Reactions were submitted to the protocol: 20°C for 10 min, 42°C for 45 min, 95°C for 5 min and 4°C for 10 min. To analyse B_1 mRNA expression, we performed real-time PCR using amounts of cDNA varying from 0.1 μg to 0.1 pg in a 10-fold scale. We carried out 25 μl reactions using 12.5 μl of the QuantiTect SYBR Green PCR Master Mix (Qiagen, Montgomery County, Maryland, USA) and 1 μl of each forward and reverse primer (18 μM each) specific for human kinin B_1 receptor mRNA (forward 5′-ACCTCAGCCTCTCGAGTTGCT-3′, reverse 5′-TGGTTGGAGGATTGGAGCTCTA-3′) or human β-actin mRNA (forward 5′-GGATCTTCATGAGGTAGTCAGTC-3′, reverse 5′- CGAGGCCCAGAGCAAGAGAG -3′). Reactions were submitted to the following protocol: 50°C for 2 min, 95°C for 10 min, and 50 cycles of 95°C for 15 s, 60°C for 20 s and 72°C for 30 s. At the end of the amplification, we conducted a dissociation protocol and submitted samples to electrophoresis in a 3% agarose gel to exclude any non-specific amplification. Fluorescence emission was measured at the end of each cycle. Ct values were plotted against logarithmic values of the amount of cDNA in order to verify PCR efficiency, and only those that fit in a linear correlation ($R^2 > 0.95$) to the cDNA quantity were considered in the analysis. We used the $2^{-\Delta Ct}$ parameter to express the relative expression data of each sample, taking β-actin as the endogenous control. As $2^{-\Delta Ct}$ has to be the same using different template amounts, we compared this parameter in every cDNA quantity used in order to obtain the experimental error. B_1 receptor mRNA expression was normalised by levels of β-actin expression within the same sample.

Reagents

CCK was obtained from Bachem (Bubendorf, Switzerland). Lys-DBK, Lys-BK, cycloheximide, CCh, KCl, atropine and indomethacin were obtained from Sigma (Varese, Italy). HOE140 and R-715 were kind gifts of Dr B. Schoelkens (Aventis, Frankfurt, Germany) and Dr D. Regoli (University of Ferrara, Italy). SR 140333, SR 48968 and SR 142801 were kindly donated by Dr X. Emonds-Alt (Sanofi-Synthelabo, Montpellier, France). Agents were dissolved in Krebs buffer, except for indomethacin, SR 140333, SR48968 and SR142801 which, at stock concentrations (10 mM), were dissolved in 100% dimethylsulphoxide (DMSO).

Statistical analysis

All data are expressed as the mean (SEM). Isometric tension data were normalised to the area of the strips, and expressed as

Table 1 E_{max} and pD_2 values of contraction induced by different spasmogens of isolated strips of control and cholecystitis human gallbladder

	First CRC (3 h)		Second CRC (6 h)	
Agonists	**pD_2**	**E_{max} (mN/mm^2)**	**pD_2**	**E_{max} (mN/mm^2)**
Control group				
CCK	7.17 (0.16)	0.46 (0.04)	7.30 (0.08)	0.47 (0.04)
Lys-DBK	6.11 (0.06)	0.04 (0.01)	5.97 (0.05)	0.18 (0.02)
CCh	5.90 (0.06)	0.10 (0.02)	5.90 (0.08)	0.12 (0.03)
Cholecystitis group				
CCK	7.58 (0.11)	0.45 (0.03)	7.53 (0.18)	0.45 (0.03)
Lys-DBK	5.93 (0.06)	0.09 (0.02)*	5.95 (0.04)	0.31 (0.04)*
CCh	5.82 (0.16)	0.11 (0.02)	5.78 (0.01)	0.10 (0.03)

The maximum effect (E_{max}) and the concentration of agonist producing half-maximum response (pD_2) were calculated on cumulative concentration–response curves performed 3 and 6 h after the in vitro incubation of tissues.
*$p<0.05$ versus the respective value of the control group.
CCh, carbachol; CCK, cholecystokin; Lys-DBK, Lys-Des-Arg^9-bradykinin.

mN/mm^2. The maximum effect (E_{max}) and the potency of the agonist, expressed as the negative logarithm of the concentration of agonist producing half-maximum response (pD_2), was obtained with an iterative curve-fitting package (Origin Software, Microcal Software, Northampton, Massachusetts, USA). Statistical analysis was performed by means of Student t test. If $p<0.05$, the results were considered significant.

RESULTS

Contraction of isolated human gallbladder strips

Gallbladder strips that did not respond to 30 mM KCl were considered not viable and were excluded from the present study. In non-precontracted strips (n = 6) or in strips precontracted with CCh (10 μM, n = 3) or KCl (120 mM, n = 4), Lys-DBK failed to cause any measurable relaxation, whereas at the highest concentrations it caused a contraction superimposed on the CCh- or KCl-induced contraction (data not shown). First and second CRCs with CCK (0.03 nM–3 μM) caused a concentration-dependent motor response in both control and cholecystitis isolated gallbladders strips, with similar E_{max} and pD_2 values (table 1). Similarly, no significant modification of E_{max} and pD_2 values of CCh-induced contractions were observed in control and cholecystitis isolated gallbladder strips in all the conditions studied (table 1). The threshold concentration for Lys-DBK to cause visible contractions of control and inflamed gallbladder strips was 30–100 nM (fig 1). Lys-DBK induced a motor response in control and inflamed gallbladder strips with similar pD_2 values (fig 1 and table 1). In contrast, the maximal contractile effect (E_{max} value) produced by Lys-DBK in the first and second CRC in gallbladder taken from cholecystitis patients was significantly higher than that observed in control tissues. In a further series of experiments, after 3 or 6 h of incubation, Lys-DBK caused a motor response with similar pD_2 and E_{max} values in specimens without the mucosa in inflamed gallbladders (table 2).

No difference has been observed between E_{max} values of Lys-DBK and CCh during the first CRC in control and cholecystitis specimens. In contrast, a statistically significant difference of two- and threefold was observed after 6 h of in vitro incubation (table 1). In addition, pD_2 and E_{max} values of Lys-DBK-induced contraction of control and cholecystitis human gallbladder strips were significantly lower than those of CCK in all experimental conditions (table 1).

In another set of experiments, the B_1 receptor antagonist, R-715 (1 μM), significantly shifted to the right the second CRC induced by Lys-DBK in cholecystitis specimens (fig 2), while the bradykinin B_2 receptor antagonist, HOE140 (1 μM), was without effect (fig 2). The combination of the tachykinin NK_1, NK_2 and NK_3 receptor antagonists, SR 140333, SR 48968 and SR 142801 (all 1 μM), atropine (1 μM) and indomethacin (5 μM) did not affect the Lys-DBK-induced contraction of cholecystitis gallbladder strips (fig 2). To confirm the co-functional expression of B_1 and B_2 receptors in the human inflamed gallbladder, the inhibitory effect of R-715 or HOE140 was studied against the prompt contractile effect induced by the B_2 agonist, Lys-BK (1 μM). The independent application of R-715 or HOE140 to the organ bath significantly, but partially, prevented Lys-BK-induced contraction (23% and 36% inhibition, respectively). In contrast, the Lys-BK-induced motor response was reduced by >70% when R-715 and HOE140 were co-administered (fig 3).

To demonstrate that the time-dependent increase in the response of cholecystitis specimens to Lys-DBK matched the increase in new protein synthesis, the protein synthesis inhibitor, cycloheximide (100 μM), was used. Cycloheximide completely prevented the time-dependent increase in the Lys-DBK-induced motor response in control and inflamed human

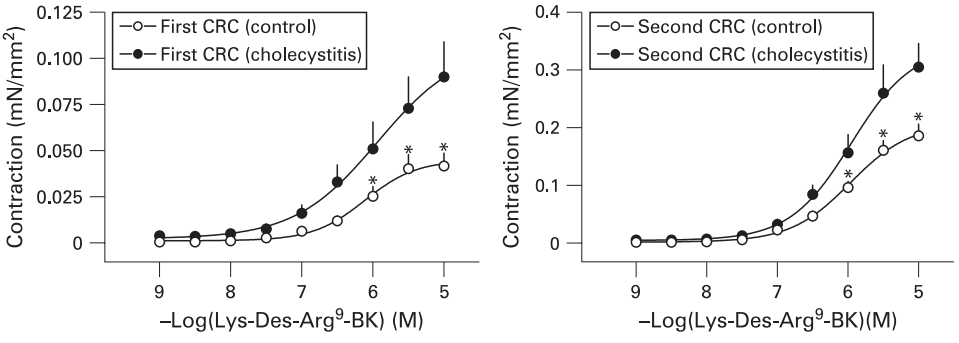


Figure 1 Cumulative concentration–response curves (CRCs) to Lys-Des-Arg^9-bradykinin in isolated strips of human gallbladder taken from control and cholecystitis patients. CRCs were performed 3 (first CRC, A) and 6 h (second CRC, B) after the in vitro incubation. Values are the mean (SEM) of at least eight experiments. *$p<0.05$ vs the respective control; Student t test.

Table 2 E_{max} and pD_2 values of Lys-DBK-induced contraction of isolated strips of mucosa-free cholecystitis human gallbladder

	First CRC (3 h)		Second CRC (6 h)	
Agonists	**pD_2**	**E_{max} (mN/mm²)**	**pD_2**	**E_{max} (mN/mm²)**
Lys-DBK	5.89 (0.14)	0.08 (0.01)	6.07 (0.12)	0.29 (0.03)

The maximum effect (E_{max}) and the concentration of agonist producing half-maximum response (pD_2) were calculated on cumulative concentration–response curves performed 3 and 6 h after the in vitro incubation of tissues.
*$p<0.05$ versus the respective value of the control group.
CRC, concentration–response curve; Lys-DBK, Lys-Des-Arg^9-bradykinin.

gallbladders after 6 h (second CRC) of in vitro incubation compared with that observed during the first CRC. In contrast, cycloheximide partially reduced, without reaching a statistically significant difference, the maximum response to Lys-DBK after 3 h of *i*n vitro incubation in both control and inflamed specimens (fig 4).

Quantitative real-time PCR

B_1 receptor mRNA levels, obtained by reverse transcription real-time PCR, were measured in the mucosa or smooth muscle layer from control and cholecystitis specimens taken immediately after surgery. A low level of B_1 receptor mRNA was observed in the mucosa of control tissues (fig 5). The slightly increased expression of the B_1 receptor mRNA did not reach statistical significance. In contrast, in the smooth muscle of cholecystitis tissues a statistically significant, threefold increase in B_1 mRNA level was observed as compared with control tissues (fig 5).

DISCUSSION

Established evidence indicates that kinins are involved in a series of physiological and pathological processes, such as control of blood pressure, contraction and relaxation of smooth muscle, the inflammatory response and induction of pain.[22] Activation of the kinin system has been demonstrated in a variety of human inflammatory diseases, such as asthma, rheumatoid arthritis, endotoxic shock, inflammatory bowel disease, pancreatitis and cystitis.[23 24] The participation of kinins in the pathophysiology of cholecystitis has been investigated by a series of experimental approaches studying the ability of bradykinin to induce motor responses in cat and guinea pig gallbladders.[25 26] Recently, we demonstrated that bradykinin induces concentration-dependent contraction of isolated strips of human gallbladders taken from control and cholecystitis patients, an effect that was significantly reduced by the B_2 receptor antagonist HOE140.[13]

In the present study, we showed that Lys-DBK, a kinin that shows high affinity for the human B_1 receptor, induces a marked concentration-dependent contraction of cholecystitis specimens with a significantly higher efficacy than that observed in control gallbladders. In addition, after 6 h of tissue incubation, Lys-DBK was more efficacious than CCh in inducing gallbladder contraction in pathological tissues. In the current study, the involvement of the B_1 receptor was assayed

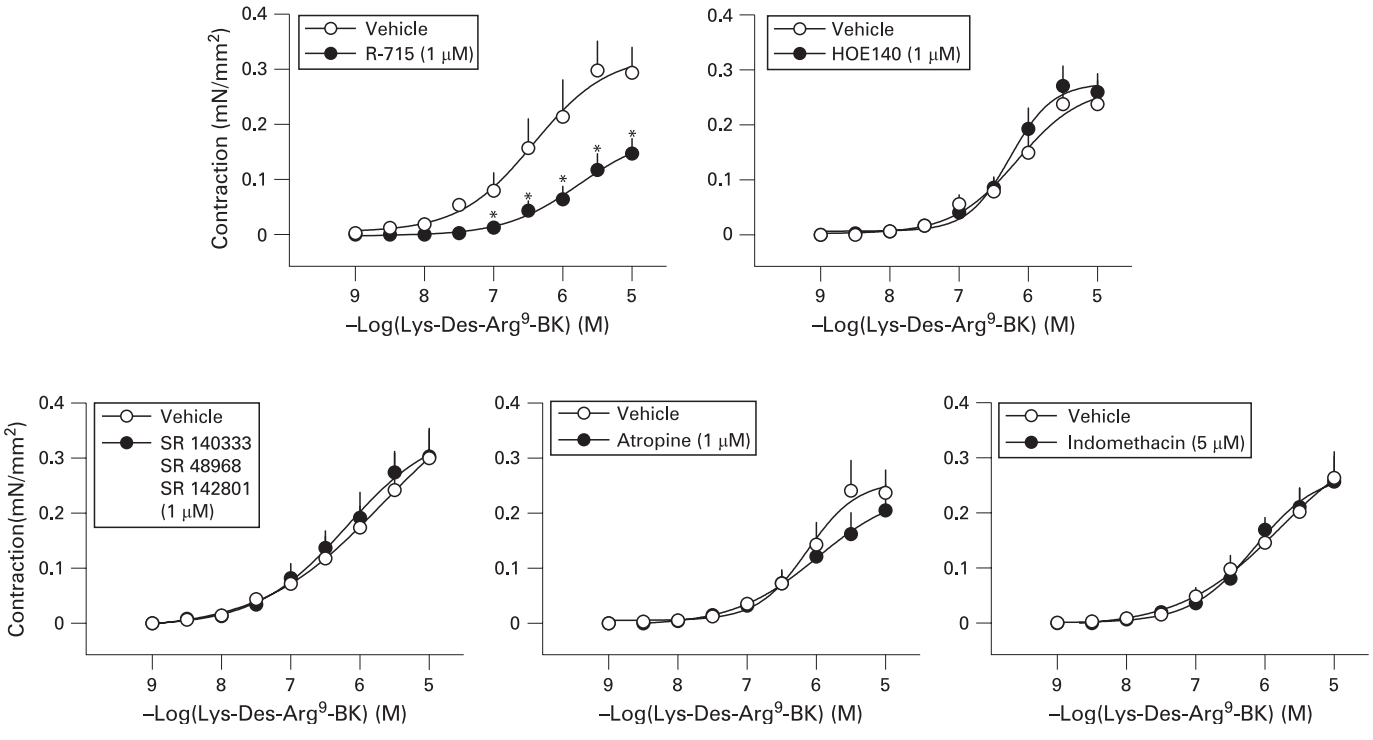


Figure 2 Effect of R-715, HOE140, SR 140333, SR 48968, SR 142801, atropine, indomethacin or their respective vehicles on the Lys-Des-Arg^9-bradykinin-induced motor responses in isolated strips of human gallbladder obtained from patients suffering from cholecystitis. Cumulative concentration–response curves were performed 6 h after in vitro incubation. Values are the mean (SEM) of at least eight experiments. *$p<0.05$ vs the respective vehicle; Student t test.

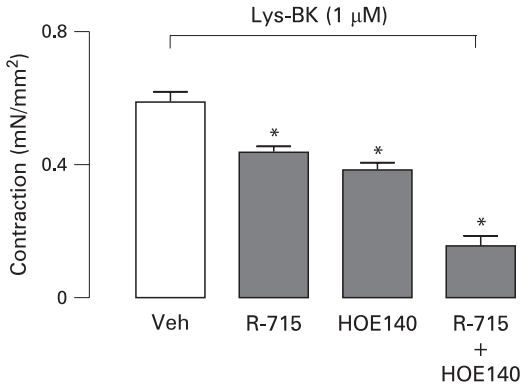


Figure 3 Effect of R-715 and HOE140 (separately or co-administered) or their respective vehicles on Lys-bradykinin (Lys-BK)-induced motor responses in isolated strips of human gallbladder obtained from patients suffering from cholecystitis. Each column represents the mean (SEM) of at least five experiments. *$p<0.05$ vs vehicle (Veh) (one-way analysis of variance followed by Dunnett test).

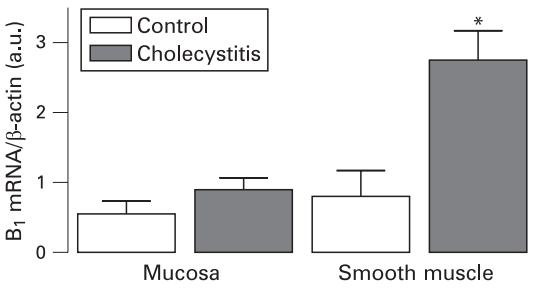


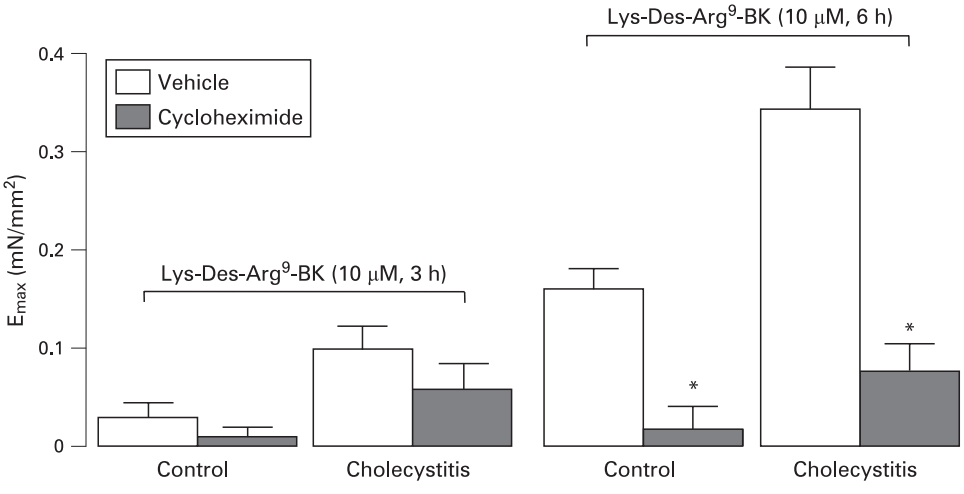
Figure 5 Real-time PCR relative quantification of kinin B_1 receptor mRNA in mucosa or the smooth muscle layer of control and cholecystitis gallbladders processed immediately after surgery. All data have been normalised by the levels of β-actin expression within the same sample. Plotted data are expressed as the mean (SEM) of specimens taken from at least four different patients. a.u., arbitrary units. *$p<0.05$ vs the respective control (one-way analysis of variance followed by Dunnett test).

by using a selective B_1 receptor antagonist, R-715, that shifted to the right the CRC induced by Lys-DBK. In contrast, the selective B_2 receptor antagonist, HOE140, did not alter the contractile effect of Lys-DBK.

Published data demonstrated that the degradation of bradykinin by endogenous carboxypeptidase may produce the weak B_1 receptor agonist, Des-Arg^9-bradykinin, that is at least 100-fold less potent than Lys-DBK on the human B_1 receptor.[27] This may explain the lack of the ability of R-715 to inhibit the contractile response induced by bradykinin in human gallbladder.[13] Thus, in order to confirm better the concomitant functional expression of both B_1 and B_2 receptors in inflamed human gallbladders, the B_2 receptor agonist, Lys-BK, which, through the catalytic action of endogenous carboxypeptidase, is transformed into Lys-DBK, a potent human B_1 receptor agonist, was used. As expected, the contraction induced by Lys-BK was only partially prevented by the independent use of the B_1 receptor antagonist, R-715, or the B_2 receptor antagonist, HOE140, whereas, the Lys-BK-induced contraction was powerfully prevented (>70% of inhibition) when both antagonists were co-administered. The present results together with our previous data[13] suggest that both B_1 and B_2 receptors appear to be expressed in the human gallbladder. Moreover, the observation that cycloheximide remarkably prevented the B_1-mediated contractile response in both control and inflamed gallbladders indicated that also in this organ new protein synthesis is the underlying mechanism for the upregulation of B_1 receptors. Failure of cycloheximide to abolish completely the contractile response to the B_1 agonist in cholecystitis specimens might be dependent on a rapid ex vivo expression of the receptor during the collection process (30–60 min). However, a substantial part of the cycloheximide-resistant contractile response is likely to be due to in vivo receptor expression because in the inflamed gallbladder.

To strengthen this hypothesis, expression of B_1 receptor mRNA was quantified by RT-PCR in the mucosa or smooth muscle layer of control and cholecystitis gallbladder specimens taken immediately after surgery. The high level of mRNA and its remarkable upregulation in colecystitis in the smooth muscle suggest that inflammation might have induced receptor expression in vivo. Thus, the present results corroborate and extend general concepts described recently[13] concerning in vitro B_1 upregulation and imply that B_1 receptors were upregulated before the in vitro incubation and this upregulation most probably depends on the inflammatory state of the tissue. Moderate B_1 receptor mRNA expression was also detected in the mucosa, with a tendency to increase in inflamed specimens. Although functional and biochemical findings indicate a prominent role in the control of human gallbladder motility

Figure 4 Effect of the protein synthesis inhibitor, cycloheximide, on Lys-Des-Arg^9-bradykinin (10 μM)-induced maximal contraction (E_{max}) in isolated strips of human gallbladder obtained from control patients and patients suffering from cholecystitis. The contractile response of Lys-Des-Arg^9-bradykinin was observed 3 and 6 h after the in vitro incubation. Cyclohexemide was maintained throughout the entire experiment. Each column represents the mean (SEM) for at least five experiments. *$p<0.05$ vs the respective vehicle (Veh) (one-way analysis of variance followed by Dunnett test).



of B_1 receptors expressed by the smooth muscle layer, a minor and indirect contribution of the B_1 receptor expressed in the mucosa cannot be completely excluded.

Upregulation of the B_1 receptor is an important feature of the kinin system during inflammation since, differently from the B_2 receptor, the B_1 receptor causes much more prolonged intracellular signals, is not desensitised and possesses high constitutive activity.[28] Thus, B_1 receptor activation has been related to the maintenance of chronic pain and inflammatory disorders. In fact, most of the literature data indicate that B_1 receptors are upregulated by several proinflammatory stimuli, including some cytokines and growth factors.[24] Interestingly, it has been shown that inflammation during gallbladder gallstone formation is associated with increased interleukin (IL) 1 levels in the guinea pig gallbladder wall.[29 30] In addition, the bile of patients with cholangitis has increased levels of the inflammatory cytokines IL6 and tumour necrosis factor (TNF) α.[31] Besides cytokines, the expression of epidermal growth factor (EGF) and its receptor was markedly increased in human chronic cholecystitis specimens.[32] It has been also demonstrated that IL1, TNFα and EGF are capable of stimulating B_1 receptor expression in several tissues.[33] Taking into account these considerations, it is tempting to suggest that some cytokines and growth factors (eg, IL1β, IL6, TNFα and EGF) could be involved in the upregulation of the gallbladder B_1 receptor, causing increased contraction in inflamed gallbladder.

We have also investigated the mechanisms involved in the contractile effect of B_1 receptor activation in human gallbladder. Some kinin actions are associated with the secondary production of other mediators including prostanoids and tachykinins.[24] However, in line with results obtained with bradykinin,[13] the contraction induced by Lys-DBK in cholecystitis gallbladders was not altered by atropine, indomethacin or the combination of NK_1, NK_2 and NK_3 receptor antagonists. Thus, these results suggest that Lys-DBK may have a direct effect on gallbladder smooth muscle or it releases mediators other than prostaglandins, tachykinins or acetylcholine. In addition, contraction of non-vascular smooth muscle induced by a B_1 agonist has been demonstrated in several isolated preparations such as rat urinary bladder and duodenum, rabbit urinary bladder human colon and pig iris sphincter.[1 34–36] In line with this evidence, it is possible to suggest that Lys-DBK produces contraction of the human gallbladder via a direct action on smooth muscle cells.

In summary, the current results expand previous data from our group[13] and reinforce the hypothesis that the kinin system plays a major role in evoking contraction in normal and, especially, in inflamed human gallbladders by stimulating both the kinin B_1 and B_2 receptors. Activation of B_1 receptors, overexpressed during the cholecystitis state, may contribute to the typical symptoms that underline biliary colic such as gallbladder painful smooth muscle contractions. The present findings also suggest that kinin B_1 and B_2 receptor antagonists may represent a valuable therapeutic option to control symptoms in patients with acute cholecystitis.

Funding: Grant support: the study was supported by a grant from MURST. JF thanks FAPERGS for the ARD fellowship. This work was partially supported by grants from the Italian Ministry of Education, University and Research (CCOFIN Project no. 2004062155 to RDeG)

Competing interests: None.

Ethics approval: The present study was approved by the Ethical Committee of the University of Ferrara.

Patient consent: All patients gave their informed consent prior to surgery.

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